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RESEARCH PAPER

Effects of freeze-thaw on soil nitrogen and phosphorus availability at the Keerqin Sandy Lands, China

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Abstract: A laboratory simulated freeze-thaw was conducted to determine the effects of freeze-thaw on soil nutrient availability in temperate semi-arid regions. Soil samples were collected from sandy soils (0–20 cm) of three typical ecosystems (grassland, Mongolian pine plantation and poplar plantation) in southeastern Keerqin Sandy Lands of China and subjected to freeze-thaw treatment (–12°C for 10 days, then 20ºC for 10 days) or incubated at constant temperature (20ºC for 20 days). Concentrations of the soil NO_3^--N, NH_4^+-N, $NaHCO_3$ extractable inorganic P (LPi) and microbial biomass P (MBP) were determined on three occasions: at the start of the incubation, immediate post-thawing and at the 10th day post-thawing. The results showed that soil net nitrification and N mineralization rates at three sites were negatively affected by freeze-thaw treatment, and decreased by 50%–85% as compared to the control, of which the greatest decline occurred in the soil collected from poplar plantation. In contrast, the concentration of soil NH_4^+-N, $NaHCO_3$ extractable inorganic P (LPi) and microbial biomass P were insignificantly influenced by freeze-thaw except that LPi and NH_4^+-N showed a slight increase immediate post-thawing. The effects of freeze-thaw on soil N transformation were related to soil biological processes and the relatively constant available P was ascribed to severe soil aridity.

Keywords: freeze-thaw; temperate semi-arid region; nitrification; phosphorus availability

Introduction

In recent years, freeze-thaw events have become increasingly concerned for their impacts on ecosystem processes, and for their frequency and intensity altered by global climate change (Murdoch & Stoddard 1998; Groffman et al. 2001). Soil freezing is a quite habitual phenomenon at middle and high latitudes. Effects of freeze-thaw on soil nitrogen (N) and phosphorus (P) transformation and their availability have been widely studied in humid regions, but rarely in arid and semi-arid regions. Previous studies indicated that freeze-thaw could stimulate soil nutrient mineralization and then increase nutrient availability (e.g. Edwards & Cresser 1992; Freppaz et al. 2007). In contrast, reduced or unchanged extractability of nutrients after freezing had also been reported (e.g. Bramley et al. 1992; Sjursen et al. 2005). The effects varied greatly with the duration, frequency and intensity of freezing, edaphic characteristics, and other factors such as vegetation type and soil moisture (Edwards & Cresser 1992).

In temperate ecosystems a significant portion (20%–70%) of annual ecosystem carbon (C) and N cycles has been found to occur in winter, and overwintering processes have great influence on subsequent plant growth (Neilsen et al. 2001). However, in northern China, although large areas of land located in temperate climate zone and soils experience a five-month winter freezing and spring thawing, ecological studies have traditionally focused on the processes occurring during the growing season, and overwintering processes have been traditionally ignored and are completely unknown. In this study, a laboratory simulated freeze-thaw treatment was conducted using sandy soils collected from three typical ecosystems (grassland, Mongolian pine plantation and poplar plantation) in southeastern Keerqin Sandy Lands in China, and soil inorganic N and labile P concentrations were determined and compared in order to understand the freezing effects on soil N and P availability in semi-arid regions.

Materials and methods

Site description and soil sampling

The soil samples used for the laboratory incubation were collected from the Daqinggou Ecological Station (42°58′N, 122°21′E, 260 m above sea level), Institute of Applied Ecology, Chinese Academy of Sciences, geographically in the southeast of

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Keerqin Sandy Lands. The area belongs to the semi-arid region in the middle temperate zone. The mean annual rainfall is about 450 mm, of which more than 60% occurs during June–August. The mean annual temperature is about 6°C (the highest 29°C in June, the lowest –30°C in January). The winter period lasts from late November to next early April, mean temperature of the surface soil during the two coldest months is about –12°C. The mean annual frost-free period is about 150 days (Zeng et al. 1996). The soil type is a coarse-textured sandy soil (classified into the Semiaripsmment taxonomic group) that is developed from sandy parent material through the action of wind (Zhenghu et al. 2007) and is deficient in N and P (Liu et al. 2002). Basic characteristics of the soil were summarized in Table 1.

Table 1. Basic soil characteristics (0–20 cm) and initial available N and P concentrations of the study sites

Site	Bulk density	pH (H_2O)	Moisture $g·kg^{-1}$	SOC $g·kg^{-1}$	Total N $mg·kg^{-1}$	Total P $mg·kg^{-1}$	NO_3^--N $mg·kg^{-1}$	NH_4^+-N $mg·kg^{-1}$	Inorganic N $mg·kg^{-1}$	LP_i $mg·kg^{-1}$	MBP $mg·kg^{-1}$
Grassland	1.5	6.8	41	6.6	333	123	0.94a	0.35a	1.28a	0.19b	2.57b
Mongolian pine plantation	1.5	6.6	34	6.5	280	90	1.21a	0.31a	1.52a	0.48a	2.74b
Poplar plantation	1.5	6.7	48	6.8	320	105	0.31b	0.22b	0.53b	0.13b	3.98a

Note: Data are means of three replicates. Values within columns with different letters were significantly different at $P < 0.05$ (LSD following ANOVA). SOC: soil organic carbon.

A natural grassland dominated by *Pennisetum flaccidum* and *Artemisia scoparia*, a 20-year-old Mongolian pine (*Pinus sylvestris* var. *mongolica*) plantation and a 20-year-old poplar (*Populus simonii*) plantation were selected as study sites. Mongolian pine and poplar were the most widely planted tree species in this region. Three 10 m ×10 m plots were established within each site for soil sampling. Plots chosen were as uniform as possible in terms of vegetation and topography. In early November 2005, 15 soil cores at 0–20 cm depth were collected randomly in each plot using an auger with inner diameter of 6 cm, and then composited into one soil sample.

Experimental treatments

A single-factor, two-treatment factorial design was used to test the effects of freeze-thaw event on soil N and P availability. Each soil sample was sieved through a 2-mm sieve to remove roots and coarse fractions (larger than 2 mm in diameter), and then divided into two sub-samples. One sub-sample was treated as the control (CK), and it was aerobically incubated at 20°C for 20 days and kept at constant field moisture by adding water everyday. The other sub-sample was subjected to freezing treatment (–12°C) for 10 days, then incubated similarly as the control for 10 days. The laboratory experiment was conducted in triplicate. Concentrations of the soil NO_3^--N, NH_4^+-N, $NaHCO_3$ extractable inorganic P (LPi) and microbial biomass P (MBP) were determined on three occasions: at the start of the incubation, immediate post-thawing of the frozen soil samples and at the 10th day post-thawing.

Soil and statistical analysis

The soil sample was extracted by 2 $mol·L^{-1}$ KCl. NO_3^--N and NH_4^+-N in the supernate were separately measured by the spectrophotometry using the cadmium reduction method and the indophenol blue method (Liu 1996). Inorganic N concentration was the sum of NO_3^--N and NH_4^+-N. Soil LPi was measured by the method developed by Bowman and Cole (1978). Soil MBP was determined by the method of chloroform fumigation extraction. The soil was fumigated with alcohol-free liquid chloroform for 24 h prior to be extracted with 0.5 $mol·L^{-1}$ $NaHCO_3$ (pH 8.5), using the efficiency factor of 0.4 to correct incomplete recovery of the microbial biomass P (Brookes et al. 1982). Soil net nitrification and N mineralization rates were calculated on the basis of 10 days. The net nitrification rate ($mg·kg^{-1}·10\ d^{-1}$) was equal to soil NO_3^--N concentration after 10 days of incubation minus the value before incubation. The net N mineralization rate ($mg·kg^{-1}10\ d^{-1}$) was equal to soil inorganic N concentration after 10 days of incubation minus the value before incubation.

One-way ANOVA was used to compare the initial available nutrients in soils. Independent-sample T test was used to determine the differences in soil properties between the control and freeze-thaw treated soil samples. Statistical processes were conducted using the SPSS statistical software package version 11.5 (SPSS Inc. 2002).

Results

Initial soil available nutrients

The initial concentrations of soil inorganic N, LPi and MBP were shown as Table 1. Concentrations of soil NO_3^--N, NH_4^+-N and inorganic N were significantly lower in poplar plantation than those in Mongolian pine plantation and grassland, but no significant difference was found between Mongolian pine plantation and grassland. LPi was significantly higher in Mongolian pine plantation than those in grassland and poplar plantations, while MBP was significantly higher in poplar plantation than those in the other sites.

Effects of freeze-thaw on the concentrations of soil available N and P

The soil NO_3^--N and inorganic N concentrations increased obviously with the increase of incubation time, while NH_4^+-N concentration was less variable than NO_3^--N during the incubation (Table 2). The soil NO_3^--N concentration at the three sites was

greatly lower in freeze-thaw treatment than in the control on all the three occasions. NH_4^+-N concentration was higher in freeze-thaw treatment than in the control immediate post-thawing in all soils, while the difference at the 10th day post-thawing was insignificant. Soil total inorganic N was always lower in freeze-thaw treatment relative to the control, but the freezing effect was insignificant in grassland and Mongolian pine plantation immediate post-thawing (Table 2).

Table 2. Available N and P concentrations ($mg \cdot kg^{-1}$) immediate post-thawing (0 day) and at the 10th day post-thawing (10th day)

Site	Treatment	NO_3^--N		NH_4^+-N		Inorganic N		LP_i		MBP	
		0 day	10th day	0 day	10th day	0 day	10th day	0 day	10th day	0 day	10th day
Grassland	CK	1.81a	3.09a	0.15b	0.27a	1.95a	3.36a	0.09a	0.17a	2.72a	2.84a
	Freeze-thaw	1.15b	2.02b	0.45a	0.24a	1.60a	2.25b	0.21b	0.18a	2.29a	2.77a
Mongolian pine plantation	CK	1.64a	3.53a	0.16b	0.27a	1.80a	3.80a	0.34a	0.33a	2.56a	2.23a
	Freeze-thaw	0.85b	2.24b	0.36a	0.25a	1.22a	2.49b	0.47a	0.48a	2.30a	2.67a
Poplar plantation	CK	1.04a	2.49a	0.16a	0.20a	1.19a	2.70a	0.13a	0.20a	3.96a	4.10a
	Freeze-thaw	0.32b	0.62b	0.26a	0.28a	0.57b	0.90b	0.19a	0.18a	3.29a	3.82a

Note: Data are means of three replicates. Values within columns for each site with different letters were significantly different at $P<0.05$ (LSD following ANOVA).

The rates of net N mineralization and nitrification in all soils decreased markedly in freeze-thaw treatment as compared with the control during the whole incubation period. Specifically, soil net N mineralization rate decreased by 53%, 58% and 83%, and soil net nitrification rate decreased by 50%, 56% and 85%, in grassland, Mongolian pine plantation and poplar plantation, respectively. Moreover the rates were obviously lower during 10 days post-thawing than 10 days of freezing (Figs. 1 and 2).

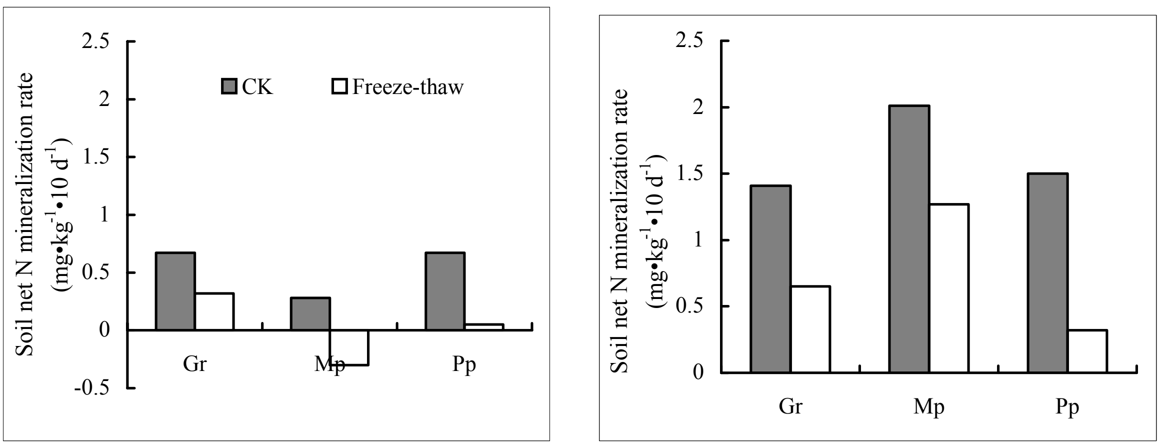

Fig. 1 Soil net N mineralization rate during 10 days of freezing (left) and 10 days post-thawing (right) at different sites. Gr, grassland; Mp, Mongolian pine plantation; Pp, poplar plantation.

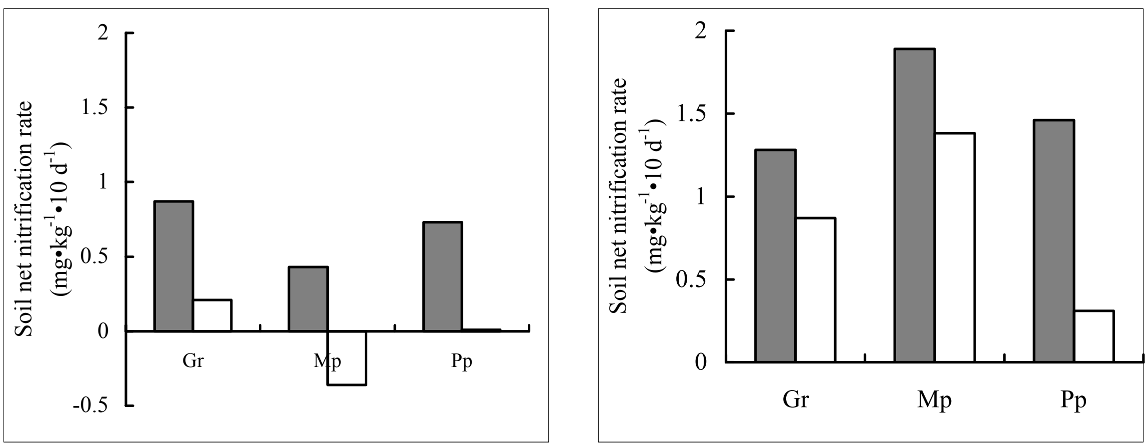

Fig. 2 Soil net nitrification rate during 10 days of freezing (left) and 10 days post-thawing (right) at different sites. Gr, grassland; Mp, Mongolian pine plantation; Pp, poplar plantation.

Effects of freeze-thaw on soil LPi and MBP concentrations were much less than those on soil inorganic N. Specifically, LPi concentration in all soil samples was higher in freeze-thaw treatment than in the control immediate post-thawing, however statistically significant difference only occurred in grassland. Soil LPi concentration in freeze-thaw treatment was almost equal to that in the control at the 10th day post-thawing. In contrast, soil MBP was not significantly affected by freeze-thaw during the whole incubation period, although it was slightly lower in freeze-thaw treatment than that in the control immediate post-thawing (Table 2).

Discussion and conclusion

Soil freeze-thaw may alter nutrient availability by disrupting physical structure of soil organic matter and by affecting microbial biomass, activity and community composition (Schimel & Clein 1996; Henry 2007). However, the overall impact and the relative importance of physical-chemical and biological processes varied with place (Edwards & Cresser 1992).

In the present study, NO_3^--N was the predominant form of inorganic N at all sites. The concentration of NO_3^--N and total N was lowest in poplar plantation, which agrees with the study by Chen et al. (2006). In all soils, both inorganic N and NO_3^--N were significantly and negatively influenced by freeze-thaw treatment, while NH_4^+-N in most soils was slightly increased by freeze-thaw treatment. These results suggested that soil N mineralization and nitrification were depressed by soil freezing, which were contrary to those obtained in most studies in humid regions (e.g. Deluca et al. 1992; Freppaz et al. 2007). Considering the special soil properties at the study sites, we speculated that biological processes associated with freeze-thaw are responsible for the depressed N mineralization. The soil at the study sites is a weakly developed sandy soil with very poor aggregate structure, low soil moisture and soil organic carbon content and low cation exchange capacity (Zhao et al. 2007). Previous studies suggested that soil moisture played an extremely important influence on the extent of soil freezing effect, because the disruption of soil aggregate structure caused by ice formation increases with soil moisture (Ron Vaz et al. 1994; Freppaz et al. 2007). Thus the effects of freeze-thaw on soil N and P availability caused by disruption of soil aggregate structure can be ignored for the low soil moisture (less than 50 $g \cdot kg^{-1}$) in winter together with very poor aggregate structure at the study sites. Therefore, decreased soil N mineralization and nitrification was mainly ascribed to the kill-off of soil microbes by soil freezing. A slight increase in soil NH_4^+-N in freeze-thaw treatment immediate post-thawing was released from the killed and lysed microbes. The effects of freeze-thaw on soil net N mineralization and nitrification were most obvious in poplar plantation (Figs. 1 and 2), possibly being ascribed to the highest soil microbial activities at this site (Table 1).

The low moisture and poor aggregate structure in soils at the study sites can also largely explain why the results in the present study differed greatly from those in most similar studies in humid regions. Disruption of ice crystals in soils breaks down soil aggregate, exposing new surfaces, particularly at high soil moisture. It makes decomposition of soil organic matter more readily, thereby stimulates soil mineralization and an increase in available nutrient concentration (Six et al. 2004), which is responsible for the increased N mineralization after freeze-thaw cycles in humid regions.

The extent of decrease in soil net N mineralization and nitrification rate in freeze-thaw treatment relative to the control was obviously smaller during the 10 days post-thawing than during 10 days of freezing (Figs. 1 and 2). The possible reason is that the soil microbes in the freeze-thaw treatment revived gradually after thawing and then increased N mineralization and nitrification rate. However, freeze-thaw effects were still significant at the 10th day post-thawing, suggesting the effects cannot be completely eliminated after thawing.

Compared with soil N transformation, less attention was focused on effects of freeze-thaw on soil P transformation in temperate regions, and there were also contradictory results (Ron Vaz et al. 1994; Peltovuori & Soinne 2005). Some studies showed that freeze-thaw treatment increased soil extractable P concentrations (e.g. Hinman 1970; Ron Vaz et al. 1994; Freppaz et al. 2007), while Sjursen et al. (2005) found a significant decline in available P and microbial P after freeze-thaw treatment. Read and Cameron (1979) reported little changes in the extractable P of soils from fall to spring. The mechanisms of freeze-thaw effects on soil P availability, similarly as that on soil N availability, involves the direct solubility of organic and inorganic compounds and breakdown of soil aggregates caused by ice formation (Freppaz et al. 2007).

In this study, being greatly different from soil inorganic N, the concentration of soil MBP and LPi was not significantly influenced by freeze-thaw treatment, just a slight increase in LPi and a slight decrease in MBP immediate post-thawing (Table 2). Low soil moisture is the main factor responsible for the relatively constant available P. Other than reducing the physical disruption of soil aggregates, low soil moisture can also directly limit the soil P transformation. Soil P transformation processes are much more sensitive to soil moisture regimes than other nutrient elements (Smith 2002). Our previous study found that organic P was the dominate form of soil P, phosphate absorbed on aluminum and iron oxides were very low (< 10% of total P), and the mineralization of organic P and turnover of microbial P were the main sources of labile inorganic P (Zhao et al. 2007). Therefore soil P transformation at the study sites is very weak and insensitive to freeze-thaw during the cold and arid winter period. Similar soil MBP concentration in freeze-thaw treatment and the control could also partly explain the less variable LPi. Moreover, low soil organic carbon content may be another contributor to the insignificant effects of freeze-thaw on soil available P according to the study by Peltovuori and Soinne (2005). Low soil organic carbon content and associated poor soil aggregate structure could greatly reduce the effects of physical disruption by soil freezing similarly as low soil moisture do. Slightly increased LPi concentration and reduced MBP in freeze-thaw treatment relative to the control immediate post-thawing can also attribute to the kill-off of soil microbes by soil freezing as discussed above.

In conclusion, soil inorganic N is negatively affected by freeze-thaw events in the semi-arid, nutrient-poor and sandy soil, while extractable inorganic P and microbial P were less affected. Additional field monitoring and more in-depth laboratory simulated experiments are needed to elucidate the mechanisms of freeze-thaw events on soil N and P transformation processes.

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